

# **EXHIBIT B**

## EDITORIAL

# The bright future of coronavirology

As a coronavirologist, I am tempted to translate the eponymic prefix as 'crown' and confer the regal attribute to my favourite viruses. However, the nomenclaturists of the International Committee on Taxonomy of Viruses probably had the *corona solis* in mind, the sunflower (*Helianthus*)—its radiating petals bearing vague resemblance with coronavirus surface projections. Coronaviruses do occupy an eminent position in virology, being not only the largest RNA viruses, but also those with the largest genomic RNA molecules known to science. The question immediately arises—why so? Or, to stay with Latin: *cui bono*—to whose advantage? To the hosts', to the viruses' survival? Is all that genetic information necessary, when other viruses with the same replication strategy can do with about one-third? What are all those extra genes for, what have they achieved in evolutionary terms?

It would be carrying owls to Athens to explain feline infectious peritonitis (FIP) to this journal's readership. Its causative coronavirus is also well known by now, thanks to studies performed mainly in Davis, Glasgow and Utrecht—the publications, however, have been largely ignored by the international community of virologists. This has changed with the emergence of SARS, coronaviruses now featuring as front-page news. The present edition of the *JFMS* contains accounts from the Second International Feline Coronavirus Symposium held last year, and it may be worthwhile to review some salient molecular and biological properties of coronaviruses, as a kind of introduction.

In the early 1980s, the *Nidovirales*, the second Order in animal virus taxonomy was defined. We had fortuitously discovered that equine arteritis virus and Berne virus, the type species of arteri- and toroviruses, respectively, use similar strategies to arrive at a 'nested set' (Latin again, *nidus* meaning the nest) of subgenomic RNAs serving messenger functions—like coronaviruses do. A nested set is a 3'-coterminal array of RNAs that are synthesized by discontinuous transcription, most likely during the

synthesis of corresponding negative-strand RNAs, which then function as templates for new plus-strands. They represent variable lengths of the 3'-end of the viral genome, each one provided at its 5'-end with a sequence identical to the genomic 5' 'leader' sequence. These mRNAs are functionally monocistronic: proteins are translated only from the most 5'-situated open reading frame. Classification of coronaviruses within the *Nidovirales* is thus based on similarities in genome organization, replication and transcription—not on virion architecture, which differs profoundly between the three. The *Nidovirales* definition actually revolutionized viral taxonomy, which had been purely structural before.

Coronavirions are spherical and contain a minimum of four structural proteins: the membrane (M), small envelope (E), spike (S) and nucleocapsid (N) protein. Trimers of the S protein form the 'peplomers', which are responsible for attachment to host cell receptors—the basis for the narrow host range of coronaviruses—and for cell-to-cell fusion.

Coronaviruses were found in all mammalian and avian species that had been examined for them. While often causing respiratory and intestinal disease, due to infection of the respective epithelia, parenchymal organs such as the liver, brain and kidneys are targets as well. Kittens are infected by their queens with a low-virulence feline enteric coronavirus (FECV) biotype that may cause a mild enteritis. The virulent FIPV biotype, which arises in individual cats by mutation, is fatal, leading to disseminated perivascular pyogranulomatous inflammation and exudative fibrinous polyserositis. Both the 'wet', effusive form and the 'dry', granulomatous form are manifestations of the same mechanism, and certainly caused by different mutants. Cells of the monocyte/macrophage lineage have been implicated as the prime targets for FIPV replication and dissemination, whereas FECV replicates primarily in mature intestinal epithelial cells. The intrinsic resistance of macrophages to

infection *in vitro* could indeed be correlated with low virulence *in vivo*.

Conceivably, the gene deletions that transform the innocuous virus into a killer must have some correlate in viral tropism; after all, FIPV is hardly transmitted, as evidenced by the sporadic occurrence of the disease. Suspected long ago, FECV can indeed persist asymptomatically, the cat's immune system being able to contain the infection. Only when the animal becomes immune suppressed, after stress, corticosteroid therapy or due to infection with, e.g., the feline leukaemia or immunodeficiency viruses, FECV replication may flare up. As a result of the expansion of the quasispecies cloud, virulent mutants may arise stochastically. In littermate studies, mutations unique to FIPV have been found in the non-essential genes 3c and, though less often, in 7b. These genes are therefore prime candidates to encode virulence determinants.

Feline medicine has not profited from these advances in coronavirus biology and pathogenesis—the diagnostics and vaccine scene has been stagnant for years, irrespective of many efforts. Progress was expected from molecular biology, but the lack of a manageable system for reverse genetics has been a frustrating experience. Because of the huge genome size, synthesis of a cDNA is an awkward, tedious process, and a different approach was badly needed. The breakthrough came three years ago, when a technique termed 'targeted RNA recombination' appeared on the scientific stage (Kuo et al., 2000). Cat aficionados will hardly believe this, but the key player is a mouse hepatitis coronavirus (MHV), which had been genetically modified to carry a chimeric S protein, with an ectodomain derived from FIPV. This virus, designated fMHV and itself generated by targeted recombination, infects cat cells but no longer those of the mouse. When synthetic RNA carrying the wild-type MHV S gene is transfected into fMHV-infected cells, recombinant viruses that acquired this gene can simply be selected on the basis of their growth in murine cells. By mirroring the method for FIPV, we selectively deleted the group-specific gene cluster ORFs 3abc and obtained deletion mutants that not only multiplied well in cell culture but also showed an attenuated phenotype in the cat. And: they protected cats against a lethal homologous challenge.

This is good news for FIP vaccinology, and there is more. In a study published last December, cats had been immunized with recombinant baculovirus-expressed N protein of a Type I FIPV; they produced homologous antibodies but, of

course, no virus-neutralizing ones. A DTH skin response to N was observed in the vaccinated cats, so cellular immunity had kicked in, and when they were challenged with heterologous FIPV, survival amounted to 75%, which is very high for this type of experiment (Hohdatsu et al., 2003). Now both vaccine approaches would need field studies to corroborate and extend the laboratory observations.

I started this editorial with the statement that coronaviruses occupy an eminent position in virology. The question about all those extra genes, probably cannibalized from host cells in the course of evolution, about their survival value will now be answered—or rather: can now be experimentally approached. They can be deleted, duplicated, inserted and reshuffled at will, and the effects of these modification can be assessed. The experiments will provide insight into pathogenesis and protection, thereby serving both biology and medicine. Coronavirology has never looked better.

## References

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## SIFFS proceedings

In 2002 veterinarians and scientists from around the world met in Scotland to discuss feline coronavirus (FCoV) and feline infectious peritonitis (FIP). The symposium brought together all the major workers in this field and united them in the fight against this lethal disease of cats. This was the second such symposium, the first having been held at the University of California, Davis, in California, in 1994. The proceedings from the conference are published in this edition of JFMS.

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- Cave et al. Risk factors for feline coronavirus seropositivity in cats relinquished to a UK rescue charity. *Journal of Feline Medicine and Surgery* 6, 53–58.
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- Pedersen et al. Common virus infection in cats, before and after being placed in shelters, with emphasis on Feline Enteric Coronavirus. *Journal of Feline Medicine and Surgery* 6, 83–88.

Although attendees paid a subscription, the conference would not have been possible without the very generous aid of the sponsors:

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I would like to take this opportunity to thank the sponsors and the attendees, who made the conference not only extremely informative, but also an event of great pleasure by creating a wonderful atmosphere. I would like to thank Prof Niels C. Pedersen, for his constant encouragement and support throughout. I am grateful to those who kindly agreed to chair a session: Dr Jim Richards, Dr Danielle Gunn-Moore, Prof Peter Rottier, Dr Raoul de Groot and to Professors Saverio Paltrinieri and Niels Pedersen for joining me in chairing the workshops at the end. Concorde Services did a great job of handling the logistics of the conference, especially Michelle Kane, whose first conference it was to organise! The Royal Academy of Music and Drama were wonderful hosts and provided delicious meals. We are very grateful to Dr Mochizuki for funding a gala dinner. Last, but by no means least, these proceedings have been made possible not just by the hard work of the authors, but also by Dr Andrew Sparkes and Marilyn Peters who have toiled over the last year to have them refereed and edited and brought to the superb state you see before you—thank you Andy and Marilyn!

The conference website, [www.felinecoronavirus.com](http://www.felinecoronavirus.com), remains up and running and will be a site for news of future symposia.

Dr Diane D. Addie

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